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LACK OF CORRELATION IN THE INTERFERON-INDUCED SMALL Mw 2-5A SYNTHETASE mRNA LEVELS AND SENSITIVITY OF TWO L CELL SUBLINES TO THE ANTIVIRAL AND PRIMING EFFECTS OF INTERFERON

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Summary. — MuIFN-alpha/beta at a concentration of 0.5 IU/ml exerted both antiviral and priming effects in the B, but not in the M subline of L929 cells. This concentration of IFN did not affect the small size 2-5A synthetase mRNA levels in either sublines, as determined by a Northern blot technique. Pretreatment with 5 or 50 IU/ml IFN resulted in more pronounced antiviral and priming effects in the L929B than in the L929M cells. However, the enhancements of the 2-5A synthetase mRNA levels due to the pretreating IFN dose were similar in both sublines.

Key words: *MuIFN-alpha/beta*; *small Mw 2-5A synthetase*; *antiviral effect*; *priming effect*

The role of 2-5A synthetase in the antiviral effect of IFN has been suggested (Williams *et al.*, 1979; Hovanessian and Wood, 1980) or shown to be unlikely (Hovanessian *et al.*, 1981; Munoz and Carrasco, 1984) in different cell-virus systems. Few data are available on its participation in the other biological activities of IFN, such as the priming effect (Content *et al.*, 1980; Lengyel, 1982; Marcus, 1984). Since we recently isolated two L929 cell sublines with different susceptibilities to the antiviral and priming effects of IFN (Rosztóczy *et al.*, 1986), it seemed of interest to study whether the inducibility of the 2-5A synthetase gene runs in parallel with the development of these biologic activities, i.e. the antiviral resistance and the primed state of cells evoked by IFN treatment. Using a Northern blot technique (Maniatis *et al.*, 1982) for the determination of 2-5A synthetase mRNA levels, we found a similar dose-response relationship in the two L cell sublines, in spite of the approximately one order of magnitude difference in their sensitivities to the antiviral and priming effects of IFN (Fig. 1). These results suggest that it is unlikely that the 2-5A synthetase whose mRNA was recognized by the probe used plays a crucial role in the mechanism of the antiviral or priming effects of IFN in the L929 cell sublines investigated by us.

The L929M and L929B cell sublines were grown in 75 cm^3 plastic tissue culture flasks. Cells were pretreated for 10 hr with different concentrations of MuIFN-alpha/beta, spec. act. $10^{7.2}$ IU/mg protein. The total cellular RNA was extracted with the phenol-chloroform extraction method (Content *et al.*, 1981; 1982) and following glyoxalization, the RNA samples were electrophoresed on 1.1% agarose gel. Blots were made on Hybond (Amersham) nylon membrane and hybridized with a random primer labelled mouse 2-5A synthetase gene probe prepared from

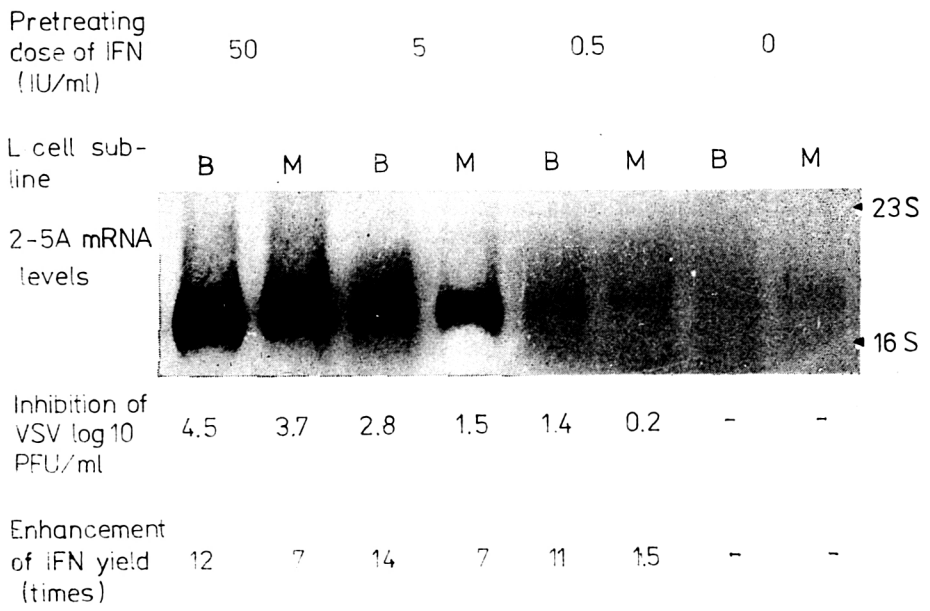

Fig. 1
Comparison of 2-5A mRNA synthetase levels, inhibition of VSV replication and priming of Sendai virusinduced IFN production in response to IFN

mouse cDNA J2 (Saunders *et al.*, 1985). For determination of the inhibition of VSV, cultures were incubated with 0.1 PFU/cell VSV and yields were determined 30 hr after infection, by plaque titration on primary chicken embryo fibroblast cells. For induction, 1.5 ml of 1.8×10^3 HAU/ml purified Sendai virus (parainfluenza 1) was added to each flask for 1 hr. Then, after removal of the inducer virus, cultures were further incubated in a 5% CO_2 in air atmosphere at 37 °C and samples were taken for IFN titration at 6 hr postinduction.

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